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ABSTRACT The development of the turkey and chicken embryo from the first cleavage division through hypoblast formation is described. The early development of the chicken embryo has been categorized into 14 stages. A similar staging sequence for the turkey was not proposed until 1993, when we described the early development of the turkey embryo, which was divided into 11 stages. Comparatively, differences in the temporal and spatial development of the turkey and chicken blastoderm were evident. Of significance is the observation that at oviposition the turkey is in Stage VII and characterized by the first signs of area pellucida formation. In contrast, the chicken embryo at oviposition is in Stage X and area pellucida formation is completed. Similarly, the hypoblast, which is already apparent in the Stage X chicken embryo, does not appear in the turkey embryo until the egg is incubated. Furthermore, the anterior-posterior (head-tail) axis in the early embryo is achieved prior to oviposition in the chicken but after the onset of incubation in the turkey. It is apparent that the turkey embryo is less mature than the chicken embryo at oviposition. Whether this distinction is related to differences between the hatchability of turkey and chicken eggs is not yet known.

(*Key words*: embryogenesis, morphogenesis, blastoderm, epiblast, hypoblast)

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INTRODUCTION

One significant problem facing the turkey industry is hatchery losses. Based on data collected for 1994 by the Economic Research Service of the USDA (Anonymous, 1995), about 412 million turkey eggs were set by commercial hatcheries in the U.S., whereas 317 million poults were actually placed. About 95 million eggs (23%) either failed to hatch or produced weak poults. Assuming hatched poult losses at 2.5%, then hatchability was about 79%.

Little information is available on the magnitude of early embryonic mortality in turkey, which may possibly be due to the difficulty in differentiating an infertile germinal disc (IGD) from an early dead blastoderm. Typically, hatcheries candle eggs after 10 to 14 d of incubation. Those eggs showing no signs of embryonic development are classified as infertile and referred to as clear eggs. These data provide the basis for candling fertility. It is only when the clear eggs are broken out and examined that the rate of early embryonic mortality can be determined, thus establishing true fertility.

Although Hodgetts (1991) suggested that 2% of the eggs set are lost due to “early dead germs”, Krueger (1990) presented data that clearly illustrated that early embryonic mortality, which was defined as embryonic death within the first 7 d of incubation and represented the difference between candling fertility and true fertility, can range between 2 and 12% during 20 wk of egg production. Likewise, 1994 data from another commercial strain (Anonymous, personal communication) indicated early embryonic mortality ranged between 4 and 14% of the eggs set. These data and those of Krueger (1990), which revealed the magnitude of early embryonic mortality of some commercial breeder flocks, suggest that early embryonic mortality is a significant problem confronting the turkey industry. Furthermore, the impact of “weak germs” as described for chickens (Summer/Fall — Hatchery Breeder Tip 1990; Mac Associates, Columbus, OH 43221) on subsequent embryonic development and poult quality can only be speculated.

In their landmark work, Eyal-Giladi and Kochav (1976) categorized the early development of the chicken embryo, which was previously inclusive of Stage 1 by Hamburger and Hamilton (1951), into 14 stages from cleavage through hypoblast formation. [For clarity, Eyal-Giladi and Kochav (1976) used Roman numerals to distinguish their stages from that of Hamburger and Hamilton, who used Arabic numerals.] More recently, it was found that temporal as well as spatial differences existed between the early morphogenetic development of the turkey and chicken embryo and, consequently, the Eyal-Giladi and Kochav (1976) procedure was not applicable to the turkey. The progressive development of the turkey embryo from cleavage through hypoblast

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formation was subsequently described (Gupta and Bakst, 1993).

The following review will describe and contrast the early morphogenetic development of the turkey and chicken embryo. The primary objective is to introduce the basis of early morphogenetic development of the turkey and chicken embryo rather than to provide a comprehensive review of the relevant literature. The anatomical terms used in this review will conform to the nomenclature suggested in the *Handbook of Avian Anatomy: Nomina Anatomica Avium* (Baumel *et al*., 1993). If not noted in this reference, the nomenclature will follow that suggested by Eyal-Giladi (1991) or Stern (1990).

DISCUSSION

Why Stage Embryos?

The quick answer to this question is that investigators need to establish the normal course of early morphogenetic development of the chicken and turkey embryo in order to provide predictable standardized stages of development. Such knowledge can be used in, but not limited to, the following applications: to define normal vs abnormal forms of embryo development (parthenogenesis); in fresh egg break outs, to differentiate blastoderms (fertile) from IGD; to evaluate the effects of farm-hatchery egg storage conditions on preincubation development and subsequent hatchability; to evaluate hen age, strain, oviposition time, and shell quality in relation to blastoderm development at oviposition, after egg storage, and incubation; to determine whether the actual morphological appearance of the IGD is reflective of the basis for the infertility, i.e., whether infertility is due to a male or female problem(s); and to determine the comparative role and function of the morphogenetic processes on further embryonic development and survival.

Early Morphogenesis of the Chicken

In their landmark work describing the morphogenetic development of the chicken embryo, Eyal-Giladi and Kochav (1976) identified three morphogenetic periods: cleavage, formation of the area (a.) pellucida, and formation of the hypoblast. Eyal-Giladi and Kochav noted that individual stages were defined based on morphological criteria and not hours of development. Furthermore, the times provided were approximations and not constant within each stage.

The cleavage period includes Stages I to VI (Figures 1 and 2), which temporally cover the initial 10 to 11 h the egg mass is in the uterus (about 6 to 16 h postovulation).

Stage I. Egg masses have either just entered or resided in the uterus for about 1 h. The blastoderm, which is 3.5 to 4.0 mm in diameter, is characterized by several cleavage furrows with nearly all cells remaining open at their periphery. The lateral surfaces of one or two cells more centrally located may be closed. Cleavage furrow formation is not always symmetrical and large vacuoles are present in the blastoderm. Minute "knobs" varying in size are observed around the periphery of the blastoderm and thought to be due to the presence of supernumary sperm. Cells on the ventral surface are open.

Stage II. After about 2 h in the uterus, a cluster of 14 to 16 blastomeres with closed lateral surfaces is observed. The lateral surfaces of the peripheral blastomeres are open and the cleavage furrows spread in all directions. Vacuoles remain in the uncleaved cytoplasm of the blastoderm. As in Stage I, cells viewed from the ventral of surface of the blastoderm remain open.

Stage III. After about 3 to 4 h in the uterus the blastoderm consists of 80 to 90 blastomeres with closed lateral sides. The overall diameter of the blastoderm has decreased and the cleavage furrows of the more peripheral blastomeres are observed extending to the

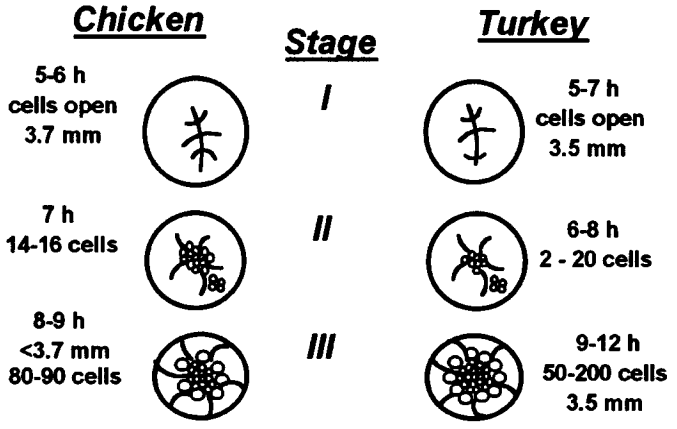

FIGURE 1. Representations of blastoderms depicting the developmental Stages I to III according to Eyal-Giladi and Kochav (1976) for the chicken and Gupta and Bakst (1993) for the turkey. Stage II blastoderms in both species often show cells or cleavage furrows in the periphery of the blastoderm.

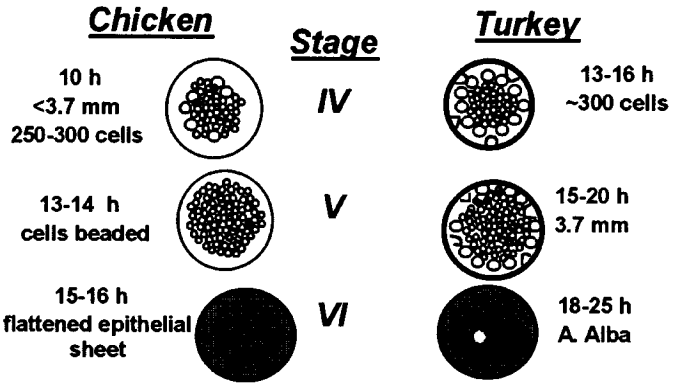

FIGURE 2. Representations of blastoderms depicting the developmental Stages IV to VI according to Eyal-Giladi and Kochav (1976) for the chicken and Gupta and Bakst (1993) for the turkey.

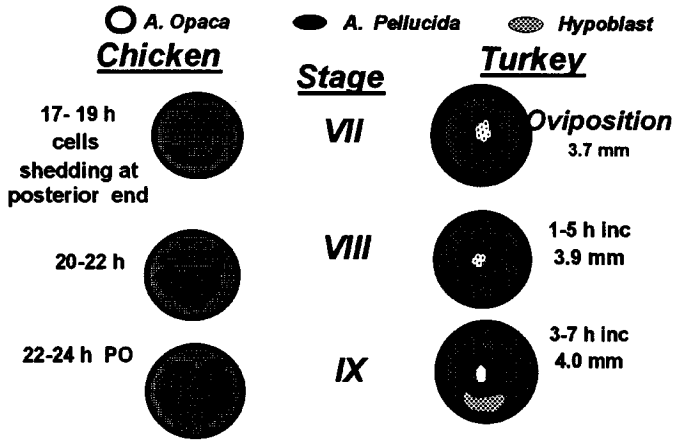

FIGURE 3. Representations of blastoderms depicting the developmental Stages VII to IX according to Eyal-Giladi and Kochav (1976) for the chicken and Gupta and Bakst (1993) for the turkey. See Also Figure 6 for another perspective of the Stage VII turkey blastoderm. PO = postoviposition; inc = incubation.

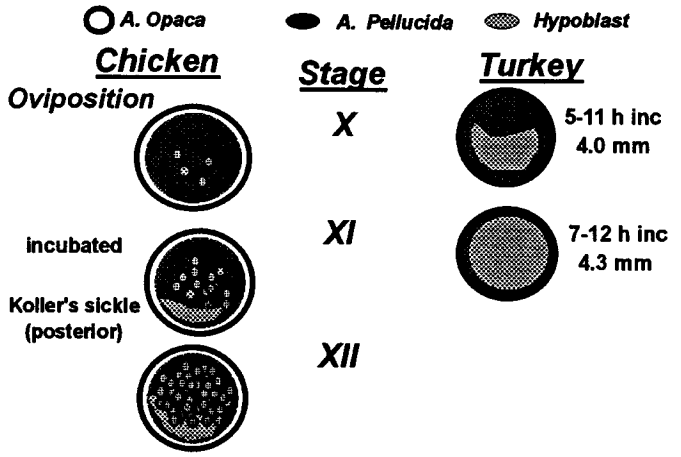

FIGURE 4. Representations of blastoderms depicting the developmental Stages X to XII according to Eyal-Giladi and Kochav (1976) for the chicken and Stages X and XI according to Gupta and Bakst (1993) for the turkey. See also Figure 7 for another perspective of the Stage X chicken blastoderm. inc = incubation.

edge of the blastoderm. About 10 to 16 closed cells are observed on the ventral surface of the blastoderm for the first time. In addition, small vacuoles are observed in the uncleaved cytoplasm. Eyal-Giladi and Kochav (1976) noted that concomitant with the closure of the cells forming the ventral surface of the blastoderm, a small centrally located subgerminal cavity is formed.

Stage IV. After about 5 h in the uterus, the blastoderm consists of 250 to 300 closed blastomeres on its dorsal surface and 80 to 90 closed cells on its ventral surface.

Stage V. After 8 to 9 h in the uterus, the blastoderm consists of closed, bead-like blastomeres that occupy equally large areas on both the dorsal and ventral surfaces. The sub-blastodermic cavity, which is referred to by Eyal-Giladi and Kochav (1976) and assumed to be the same as the subgerminal cavity observed in the previous stage, has increased in size.

Stage VI. By 10 to 11 h in the uterus (about 16 to 17 h postovulation), the dorsal and ventral surfaces of the blastoderm consist of small cells that form a uniformly thick epithelial layer.

The period that coincides with the formation of the a. pellucida includes Stages VII to X (Figures 3 and 4), which temporally covers the last 8 to 9 h the egg mass is in the uterus (about 17 to 25 h postovulation).

Stage VII. After 12 to 14 h in the uterus, the cells that form the dorsal surface of the blastoderm continue dividing and appear to be reduced in size. In contrast, the cells on the ventral surface of the blastoderm are about the same size as they were in Stages V to VI. The first evidence of the a. pellucida is noted as a more transparent area in the posterior half of the blastoderm. This thinning of the posterior aspect of the ventral surface of the blastoderm is attributed to cell shedding. The shed cells are observed resting on the yolk surface at the lower face of the subgerminal cavity. This morphogenetic process establishes the anterior-posterior axis of the embryo.

Stage VIII. After 15 to 17 h in the uterus, a. pellucida formation has progressed and is manifested as a transparent sickle-shaped area when viewed dorsally. In contrast, the periphery of the blastoderm remains unchanged and this margin forms the a. opaca. Large bead-like cells on the ventral surface of the blastoderm appear to shed.

Stage IX. After 17 to 19 h in the uterus, the transparent region forming the a. pellucida, although still incomplete, has progressed more in an anterior direction. As a. pellucida formation remains incomplete, the a. opaca is not clearly delineated in the anterior portion of the blastoderm.

Stage X. After 20 h in the uterus, the egg is oviposited. In the fresh laid egg, a. pellucida formation is completed and it is clearly delineated from the a. opaca, which marked the peripheral border of the blastoderm. The initial indications of hypoblast formation are observed in this stage. Clusters of small cells are observed forming a meshlike layer at the posterior region of the blastoderm (Figures 5 and 6). A sickle-shaped belt remains at the posterior margin of the a. pellucida, which indicates that this new layer of cells does not extend to the a. opaca.

The final period of morphogenetic development, that of hypoblast formation, is associated with Stages XI to XIV (Figures 4 and 7) and only advances upon incubation.

Stage XI. Observations through the nearly transparent a. pellucida reveals deeper concentrations of cells, which are better resolved when viewed from the ventral surface. The inner aspect of the a. opaca is highlighted by a narrow transparent band (presumptive marginal zone), the anterior border of which is marked by an accumulation of cells arranged in an arc-like manner, possibly representing Koller's sickle. If present, Koller's sickle marks the posterior boundary of the future hypoblast.

Stage XII. The transparent belt at the posterior aspect of the a. opaca remains and the hypoblast lines about half of the ventral surface of the a. pellucida. The hypoblast

appears to be formed by the multiple fusion of separate cell masses.

Stage XIII. The transparent belt at the posterior aspect of the a. opaca remains evident as hypoblast formation is completed. No invaginations or depressions are evident on the epiblast.

Stage XIV. The anterior aspect of the hypoblast is well-defined and the posterior aspect of the hypoblast and the a. opaca forms a cellular bridge, an event immediately preceding primitive steak formation (Stage 2: Hamburger and Hamilton, 1951).

Early Morphogenesis of the Chicken: More Recent Observations

In a comprehensive review article, Eyal-Giladi (1991) summarized and further elaborated upon observations of early chick development made after the Eyal-Giladi and Kochav (1976) publication. As it is beyond the scope of this work to do a similar comprehensive review, the following section will both highlight new information on the morphogenetic development of the early chick embryo and address some of the contentious issues that remain.

Eyal-Giladi (1991) noted that during Stages I to III no nucleoli are observed in the blastodermal cell nuclei. It is not until Stage VI that two or more "pronucleoli" are observed, which suggests that no rRNA synthesis was occurring during the cleavage stage. Mature nucleoli, which are assumed to be capable of synthesizing rRNA, did not appear until Stage VII. More recently, Hargroove (1993) confirmed that the switch from maternal to embryonic control is observed between Stages VI and VII. Eyal-Giladi (1991) also noted that the Stage V blastoderm, which was five to six cells thick and radially symmetrical, is marked by an accumulation of intracellular glycogen.

During the period of a. pellucida formation, Eyal-Giladi (1991) noted that the five to six cell thick central area of the ventral surface of the Stage VII blastoderm begins to thin until, by Stage X, that portion of the blastoderm overlying the subgerminal cavity is only one cell thick. This process excludes the outermost peripheral cells, which thereafter form the a. opaca. The formation of the a. pellucida (Figure 3) was considered to be the first morphogenetic event in avian embryo development (Eyal-Giladi, 1991). Additional data is reviewed by Eyal-Giladi (1991), which show that cell shedding during the formation of the a. pellucida actually is a result of the loss of cells as manifested by a 75% reduction in the area of the blastoderm (cytoplasmic area) between Stages VI (the onset of cell shedding) and Stage X.

It is of particular interest that Eyal-Giladi and Spratt (1965) observed that the stage of blastoderm development in the unincubated egg varied considerably (from a single layer to primitive streak formation) due to environmental temperature. Notwithstanding this observation, a freshly laid egg should be at Stage X with few, if any, nonshed cells associated with the anterior portion of the a. pellucida. Close examination of the ventral surface of the

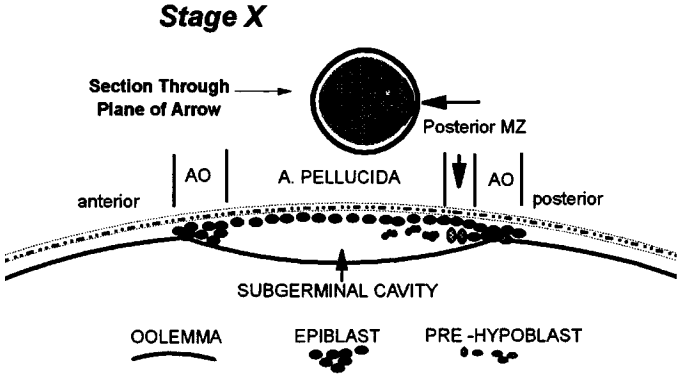

FIGURE 5. A midsagittal section through the Stage X chicken blastoderm depicting the spatial organization of the embryo at the time of oviposition. AO = area opaca; MZ = marginal zone.

a. pellucida of the Stage X blastoderm revealed single cells and clusters of cells either derived through polyingression or polyinvagination (Figure 5). Upon incubation, these cells will, in part, constitute the primary hypoblast (defined below).

The Stage X embryo consists of an a. pellucida that has not differentiated into an epithelial-like epiblast (Watt *et al.*, 1993). Watt *et al.* further suggested that such undifferentiated cells may be the basis of successful chimera formation following the transfer of Stage X donor cells to Stage X recipient embryos. [One of the Watt *et al.*, (1993) authors (R. Etches, University of Guelph, personal communication), commented after the presentation of this work at the Symposium that, although not differentiated, the epiblast cells may already be "determined" by Stage X.]

Eyal-Giladi (1991) further subdivided the a. pellucida of the Stage X blastoderm into two regions, which she indicated are difficult to define with certainty. These regions are the central disc (CD) of the a. pellucida and the marginal zone (MZ) (Figure 5). The CD is apparent if the sickle-shaped belt of cells at the posterior margin of the a. pellucida (Koller's sickle) is discernible. The narrow, epiblastic band between Koller's sickle and the inner aspect of the a. opaca represents the MZ, and this MZ surrounds the CD. These structures may or may not be discernible in the freshly laid, unincubated egg, but are clearly discernible by Stage XIII.

Stern (1990) and Eyal-Giladi (1991) differ in their morphological assessment of the MZ. Eyal-Giladi (1991) indicated that the ventral surface of the MZ is often accompanied by a shelf-like extension of the a. opaca (Figure 6) assumed to be part of the germ wall (for explanation, see Romanoff, 1960). Stern (1990) further elaborated on the cell composition of the posterior MZ and suggested that it is composed of at least two distinct cell types. The upper, one cell thick epithelial layer of the posterior MZ is continuous with the epiblast forming the a. pellucida and a. opaca and contributes cells to the superficial aspect of the primitive streak and later to the definitive gut endoderm. The lower layer, which covers

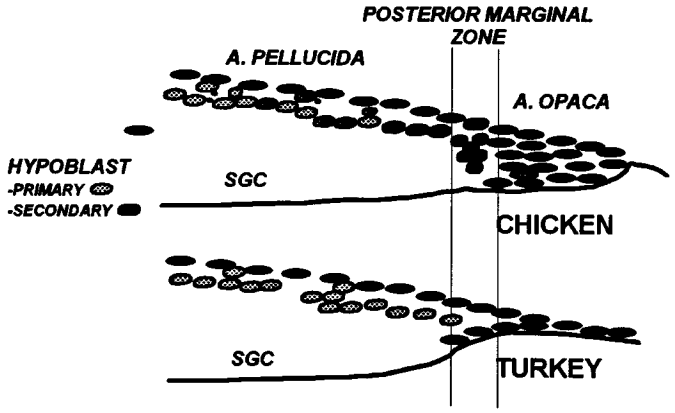

FIGURE 6. Comparative representations of the posterior region of the chicken and turkey blastoderm around the time of completion of the hypoblast. As depicted, it has yet to be determined whether hypoblast formation in the turkey embryo is similar to that of the chicken embryo. The germ wall, which is formed by those cells in the region of the a. opaca, is more attenuated at the periphery in the turkey blastoderm compared to the chicken blastoderm. The subgerminal cavity (SGC) is also shown.

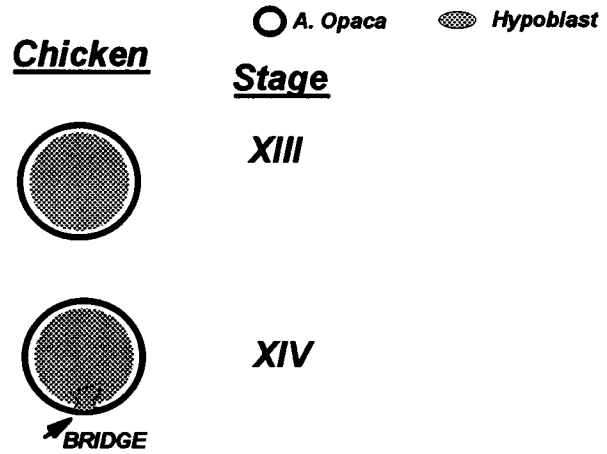

FIGURE 7. Representations of blastoderms depicting the developmental Stages XIII to XIV according to Eyal-Giladi and Kochav (1976) for the chicken. The cellular bridge connects the a. opaca with the hypoblast at the posterior end of the blastoderm.

the ventral surface of the upper layer of the posterior MZ (Figure 6), is continuous with the large yolk-laden cells of the deep a. opaca endoblast (also referred to as the germ wall). Stern (1990) indicated that these cells, which constitute a heterogenous population, contribute to "secondary" hypoblast formation ["secondary" hypoblast as defined by Stern differs from that defined by Eyal-Giladi (1991, see below)].

Eyal-Giladi (1991) defined the primary hypoblast as that layer subjacent to the epiblast that is formed in a posterior-to-anterior direction between Stages X and XIII, before the addition of ectodermal cells. According to Eyal-Giladi (1991), the primary hypoblast is derived from two cell populations, one by polyingression or polyinvagination of the epiblast cells, and the other from Koller's sickle, or more generally, the posterior end of the blastoderm. Although polyingression is observed by Stage X, cell migration from the posterior end is first observed in the Stage XI blastoderm. By Stage XIII, the cells comprising the completed primary hypoblast, which form a continuous layer subjacent to the CD of the a. pellucida, are joined by tight junctions. In contrast to Stern (1990, see above), Eyal-Giladi (1991) defined the secondary hypoblast as a region in the primitive streak stage of development into which definitive endoplastic cells have penetrated. Unfortunately, standardized nomenclature such as that provided by the *Handbook of Avian Anatomy: Nomina Anatomica Avium* (Baumel *et al.*, 1993) has not been adapted to early morphogenetic processes. Consequently, the clusters of cells on the ventral surface of the epiblast observed between Stages XI and XII have been referred to as the endophyll (Vakaet, 1970), polyinvaginated or polyingressed cells (Eyal-Giladi and Kochav, 1976; Eyal-Giladi, 1991), and the primary hypoblast (Stern, 1990).

Watt *et al.* (1993) using scanning and transmission electron microscopy, could not definitively discern cell shedding from polyingression of cells comprising the epiblast. Furthermore, in contrast to previous observations (see above), Watt *et al.* (1993) noted that the epiblast of the Stage X embryo is comprised of three to four cell layers along with the clusters of cells that forms the primary hypoblast. However, by Stage XII the columnar epithelium forming the epiblast is only one cell thick.

By Stage XII, cells derived from Koller's sickle and the polyingressed cells on the ventral surface of the epiblast had begun to become confluent. The sheet-like product of this process was referred to as the primary hypoblast by Eyal-Giladi and Kochav (1976). In contrast, Stern (1990) referred to the polyingressed cells on the ventral surface of the epiblast as the primary hypoblast and the first cells emerging from the deep layers of the posterior margin in the posterior part of the embryo as the secondary hypoblast. Stern (1990) noted that the cells of the primary and secondary hypoblast form the hypoblast sheet. Noting such differences in nomenclature, Eyal-Giladi (1991) suggested that the term primary hypoblast be used to describe the forming lower layer up to Stage XIII and prior to the penetration of definitive ectodermal cells. And, as noted previously, Eyal-Giladi (1991) suggested that the term secondary hypoblast be limited to the lower layer at primitive streak stages, into which definitive endoplastic cells have already penetrated. Notwithstanding, Stern's (1990) nomenclature was adapted by Watt *et al.* (1993).

In the Stage XIII blastoderm, a narrow space, the blastocoele (in contrast to subgerminal cavity, which was bounded by the ventral surface of the blastoderm and the oolemma overlying the yolk) separates the two germ layers, the epiblast and hypoblast. Furthermore, the a. opaca, MZ, CD of the a. pellucida, and the hypoblast forming the Stage XIII blastoderm, which can rightly be referred to as a blastula, have divergent developmental fates.

Early Morphogenesis of the Turkey

In contrast to the body of knowledge available on the early morphogenetic development of the chicken embryo, little is available with regard to the early development of the turkey embryo. After realizing that the chicken embryo staging procedure was not totally applicable to the turkey embryo, Gupta and Bakst (1993) defined 11 stages of turkey embryo development from cleavage through completion of the hypoblast.

In the following section, three periods of morphogenetic development are described and include the oviducal period, oviposition (and when applicable, cool egg storage), and incubation. Following the standard of Eyal-Giladi and Kochav (1976), individual stages were defined based on morphological criteria and not hours of development. The times provided for stages in the oviducal and incubation periods were approximations and not constant within each stage.

The oviducal period included Stages I to VI (Figures 1 and 2) and is characterized by cleavage and subsequent cell size reduction, cell proliferation, and subgerminal cavity formation. The time estimates provided assume 6 h between ovulation and the entrance of the egg mass into the uterus.

Stage I. Egg masses are enveloped by a shell membrane and are estimated to be in the oviduct 4.5 to 6.5 h. The dorsal surface of the blastoderm is characterized by the asynchronous and asymmetric formation of cleavage furrows. Blastomeres are open at the periphery but sometimes form a single central cell. No cleavage furrows are observed on the ventral surface of the blastoderm. Vacuole formation is only evident on the dorsal surface after the blastoderm has been in a saline buffer for longer than 30 min.

Stage II. The egg masses were in the uterus up to 2.5 h. Cleavage furrows have increased in number and form a central cell mass consisting of 2 to 20 blastomeres. This central area of cells was surrounded by larger open cells with furrows that often extended to the periphery of the blastoderm. Small clusters consisting of accessory cleavage furrows and occasionally up to six cells, are observed in the peripheral regions of some blastoderms. These are only observed in Stages II and III and may represent sites where accessory sperm nuclei have activated the ooplasm thus initiating cleavage. The appearance of the ventral surface is similar to that of Stage I.

Stage III. After 3 to 6 h in the uterus, the dorsal surface of the blastoderm is organized into three areas: a central cell mass of about 50 to 200 smaller, rounded cells, a middle layer of large polygonal cells, and a peripheral layer of larger open cells. For the first time a few cleavage furrows and blastomeres are observed on the ventral surface. Given the presence of these cells, it is assumed that histological sections of Stage III embryos would reveal the initial appearance of the subgerminal cavity.

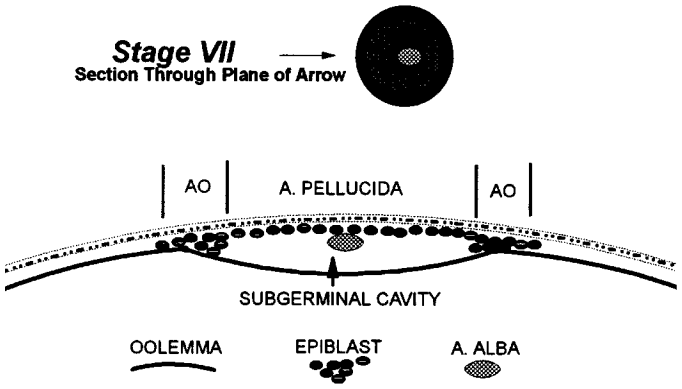

FIGURE 8. A midsagittal section through the Stage VII turkey blastoderm depicting the spatial organization of the embryo at the time of oviposition. AO = area opaca.

Stage IV. The egg mass has been in the uterus for 7 to 10 h and the blastoderm retains the basic appearance of the Stage III embryo. However, the central cells have increased in number (about 300 cells) and decreased in size while occupying a larger central area of the blastoderm. The middle and outer layers are more peripherally displaced and more attenuated than in the Stage III embryo. The number of cells on the ventral surface ranges between 20 to 200 and the lateral extent of the subgerminal cavity varies accordingly.

Stage V. The egg mass has been in the uterus for 9 to 14 h and the dorsal surface of the blastoderm is nearly totally occupied by the central cell mass. In contrast, the middle and outer layers appear to be narrow and occasionally incomplete. The ventral surface of the blastoderm consists of about 200 cells, most of which are larger than the cells that form the central cell mass. The subgerminal cavity expanded in diameter.

Stage VI. The egg mass has been in the uterus for 12 to 19 h. The central cell mass nearly covers the entire dorsal surface of the blastoderm. Near its center, the blastoderm is five to six cells thick but it is only two to three cells thick in its periphery. The central part of the blastoderm possesses an irregular shaped, small whitish area, the a. alba, which is observed for the first time. The ventral surface has increased numbers of cells compared to Stage V and the subgerminal cavity has expanded toward the periphery of the blastoderm.

The period encompassing oviposition and preset storage (cool eggs storage before incubation) spanned Stages VII to VIII (Figure 3). Unpublished observations from our laboratory indicate that eggs cooled for storage prior to incubation advance to Stage VIII before the temperature-induced developmental arrest.

Stage VII. The dorsal surface of the blastoderm from the freshly laid egg is characterized by three distinct areas: the a. opaca, which is the most peripheral area of the blastoderm and consists of a two to three cell thick layer of epiblast cells overlying yolk, the a. pellucida, which consists of a two to five cell thick layer of epiblast cells

overlying the subgerminal cavity, and the a. alba, which consists of a four to five cell thick layer of cells located in or near the center of the blastoderm (Figure 8). The same three areas are visible on the ventral surface. The area alba is characterized by large, centrally located, opaque cells.

Stage VIII. When examined during storage and after the first 5 h of incubation the a. pellucida has expanded peripherally and the a. opaca and a. alba are less prominent. The same clusters of cells are observed around the a. alba when viewed from the ventral surface.

The incubation period, Stage IX to XI (Figures 3 and 4), commences with incubation and is characterized by the initial appearance and later the completion of the hypoblast.

Stage IX. After 3 to 7 h of incubation, the a. pellucida remains about the same size as that in Stage VIII, and it and the a. opaca remain distinctive whereas the a. alba is less prominent. The hypoblast is initially observed as a faint arc occupying about 20 to 30% of the a. pellucida subjacent to the epiblast. The hypoblast establishes the posterior aspect of the blastoderm. Viewing the ventral surface of the blastoderm more clearly reveals the hypoblast as an arc-shaped compact mass of cells.

Stage X. After 5 to 11 h incubation, the central aspect of the area pellucida and about 50% of its total surface area is highlighted by a whitish region that corresponds to the nearly completed hypoblast. The a. alba is not clearly evident. When viewed from the ventral surface, the hypoblast is not yet a uniform sheet of cells.

Stage XI. After 7 to 12 h of incubation, the hypoblast is observed as a circular area in the central 50% of both the dorsal and ventral surface of the blastoderm. In this region the epiblast and subjacent hypoblast are each one to two cells thick.

Infertile Germinal Disc. In contrast to the organization observed in the blastoderm at oviposition, the IGD generally appears asymmetrical with a dense, irregular shaped, whitish central area surrounded by a variable number of vacuoles. The vacuoles are occasionally discernible upon close inspection with the unaided eye and are always observed after some magnification. Also with magnification, occasionally observed in the dense whitish area are cell-like structures of varying diameters. After 24 h (or longer) of storage at 15 to 18 C, the clearly defined vacuoles that characterized the freshly laid IGD become less apparent, rendering the differentiation of the IGD from the blastoderm more difficult. Although Eyal-Giladi and Kochav (1976) observed vacuoles on the ventral surface of Stage I to III chicken embryos, vacuoles are only observed associated with turkey embryos (up to Stage IV) after their isolation and storage in buffer for periods longer than 30 min.

Comparative Development of the Turkey and Chicken Embryo

Gupta and Bakst (1993) noted that the staging procedure developed by Eyal-Giladi and Kochav (1976) was not totally applicable to the turkey. Of the three morphogenetic periods described for the chicken embryo, cleavage (Stages I to VI) and a. pellucida formation (Stages VII to X), which includes the initial visualization of the a. opaca, were completed prior to, or soon after, oviposition (Eyal-Giladi and Kochav, 1976). These authors also noted that the diameter of the blastoderm increased progressively from Stage VII onward. In contrast, it appears that in the turkey embryo, a. pellucida formation began shortly before oviposition by some yet to be described morphogenetic process. Furthermore, unlike the chicken embryo, which spatially began a. pellucida formation in the posterior aspect of the embryo, a. pellucida formation in the turkey embryo appeared initially around the a. alba, a structure unique to the turkey embryo. Whereas Eyal-Giladi (1991) noted that formation of the a. pellucida and later, the formation of the hypoblast were temporally separate morphogenetic events in the chicken embryo, the formation of the a. pellucida and hypoblast revealed some degree of temporal overlap in the turkey embryo (Gupta and Bakst, 1993). In addition, the head-tail axis in the early embryo is achieved prior to oviposition in the chicken but after the onset of incubation in the turkey. Finally, the diameter of the turkey blastoderm increased significantly and progressively after oviposition.

It is apparent that the morphogenetic development of the chicken embryo is more advanced than that of the turkey embryo at the time of oviposition. Whether this is related to differences between the hatchability of turkey and chicken eggs is not known and currently being examined.

REFERENCES

Anonymous, 1995. Poultry Outlook—Supplement to Livestock, Dairy, and Poultry Situation and Outlook. Economic Research Service—U.S. Department of Agriculture. LDP-P-5, February 28, 1995, Washington, DC.

Baumel, J. J., A. S. King, J. E. Breazile, H. E. Evans, and J. C. Vanden Berge, 1993. Handbook of Avian Anatomy: Nomina Anatomica Avium. Publications of the Nuttall Ornithological Club, Cambridge, MA.

Eyal-Giladi, H., 1991. The early embryonic development of the chick, as an epigenetic process. Crit. Rev. Poult. Biol. 3: 143–166.

Eyal-Giladi, H., and N. T. Spratt. 1965. The embryo-forming potencies of the young chick blastoderm. J. Embryol. Exp. Morphol. 13:267–273.

Eyal-Giladi, H., and S. Kochav. 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. Dev. Biol. 49:321–337.

Gupta, S. K., and M. R. Bakst, 1993. Turkey embryo staging from cleavage through hypoblast formation. J. Morphol. 217: 313–325.

Hamburger, V., and H. L. Hamilton, 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88:49–92.

Hargroove, T., 1993. Culture and freezing of Stage X chicken embryos and the onset of embryonic transcription in the preoviposition chicken embryo. Dissertation, Department of Poultry Science, University of Maryland, College Park, MD.

Hodgetts, B., 1991. Current hatchabilities in species of domestic importance and the scope for improvement. Pages 139–144 *in*: Avian Incubation, S. G. Tullet, ed. Butterworth-Heinemann, London, UK.

Krueger, K. K., 1990. Fertility in female turkeys: How to manage it? Pages 205–211 *in*: Control of Fertility in Domestic Birds, J. P. Brillard, ed. Les Colloques de L'INRA, 54, Tours, France.

Romanoff, A. L., 1960. The Avian Embryo: Structural and Functional Development. The Macmillan Co., New York, NY.

Stern, C. D., 1990. The marginal zone and its contribution to the hypoblast and primitive streak of the chick embryo. Development 109:667–682.

Vakaet, L., 1970. Cinephotomicrographic investigations of gastrulation in the chick blastroderm. Arch. Biol. (Liege) 81: 387–426.

Watt, J. M., J. N. Petitte, and R. J. Etches, 1993. Early development of the chick embryo. J. Morphol. 215:165–182.